mix 200 gel slurry to gether.
Wash cells 2x w/ PBS
Add 4 ml media (complete w/ serum)
Add DNA o/n

I devised strategies for making the Adeno ΔE1 sp1A & 1B constructs
Basically - move in the reported gene and then PCR the minimal enhancer and move in as a second cloning.

For example: PSE driving β-gal in Adeno

orientation w/ ClaI
1. Take XbaI fragment (3.88 kb) from pCMV-β-gal which has SV40 sp/pA, β gal & polyA into XbaI vector ΔE1 sp1A & 1B

orientation w/ ClaI
2. PCR the minimal enhancer w/ HindIII ends. Note HindIII does not cut in the 3.88 kb of β-gal. Clone in the enhancer & we are done

PSE driving CAT in Adeno

orientation w/ EcoRI
1. Take XbaI/BamHI of pCAT basic (1.7 kb) and move into XbaI/BamHI of pΔE1 sp1A & 1B.

orientation w/ ClaI ± EcoRI
2. In the resultant plasmids above in HindIII ends of the minimal PSE. The same as above.... HindIII does not cut CAT & only cuts 1X in the MCS of the vectors

PSE driving DTA in Adeno

orientation w/ EcoRI
1. Take XbaI/BamHI of DT44 (1.4 kb) and clone into XbaI/BamHI of pΔE1 sp1A & 1B

orientation w/ ClaI ± EcoRI
2. Clone in minimal PSE w/ HindIII ends as above.

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Witnessed & Understood by me,
Date
Invented by D. Henderson
Date
Recorded by